Jl S. Afr. Bot. 44 (4): 317–328 (1978)

THE VASCULAR ANATOMY OF THE SEEDLING OF *ENCEPHALARTOS EUGENE-MARAISII* VERDOORN

P. J. Robbertse, S. van der Westhuizen and G. K. Theron
(Department of Botany, University of Pretoria)

Abstract

The path of the vascular strands in the seedling of *Encephalartos eugene-maraisii*, from the primary root to the cotyledons and first vegetative leaves is discussed. Each of the two cotyledons is supplied with three leaf traces while the first vegetative leaf is supplied with four vascular bundles orginating from a girdle in the vascular plate. The primary xylem in the leaf base is mesarch, becoming endarch towards the first pinna.

Uittreksel

DIE VAATANATOMIE VAN *ENCEPHALARTOS EUGENE-MARAISII* VERDOORN SAAILINGE

Die ligging van die vaatbundels in die *Encephalartos eugene-maraisii* saailinge word vanaf die primêre wortelpunt tot by die saadlobbe en eerste vegetatiewe blare bespreek. Elk van die twee saadlobbe is voorsien van drie blaarvaatstringe terwyl die eerste vegetatiewe blare van vier vaatstringe wat uit 'n gordel in die vaatplaat ontstaan. Die primêre xileem in die blaarbasis is mesarg en word endarg in die rigting van die eerste pinna.

Introduction

The Cycadales is a very interesting plant group and it is not surprising that by the end of the 19th century a considerable amount of literature had been built up on a variety of morphological aspects of this taxon. Very little, however, has been done on the South African *Encephalartos* species and therefore a morphological study of *E. eugene-maraisii* was started by van der Westhuizen (1976). During this study it became clear that there was still doubt as to the origin of the centrifugal xylem in the petiole of the Cycads in spite of Le Goc's (1914) paper which dealt with this problem. There was also doubt about the number of vascular strands entering the cotyledons of seedlings of *Encephalartos* species since Matte, according to Dorety (1908), described the anatomy of *E. barteri* with three cotyledons (probably an abnormal seedling), each with only one trace.

This study was undertaken to contribute to the knowledge of the anatomy of the Cycadales and to try to supply answers to the above-mentioned problems.

Material and Methods

Two-month-old seedlings of *Encephalartos eugene-maraisii* and of *E. transvenosus* were obtained from the Division of Nature Conservation, Transvaal

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Accepted for publication 25th November, 1977.

Provincial Administration. One seedling was transversely sectioned at the positions indicated in Fig. 1. Diagrams of the sections are shown in Fig. 2A–S. Serial microtome sections, 8–10 μm thick, were made of one other seedling and hand sections were made of two other seedlings. Standard procedures for embedding, sectioning and staining were followed to obtain serial sections (Johansen, 1940).

RESULTS AND DISCUSSION

At the seedling stage depicted in Fig. 1, one vegetative leaf has developed while the greater part of the two cotyledons is still embedded in the female gametophyte tissue which is enveloped by the seed coat. The proximal end of the primary root is tuberous, and a few young apogeotropic roots have developed (not shown in figure).

At levels A and B (Figs 1 and 2) the roots of some seedlings are octarch while others are tri- or tetrarch. In the root from which the sections A–S were prepared no endodermis was distinguishable although it was well-developed below this point in all the roots examined. The pith is well-developed and its diameter increases in the tuberous part of the root, due to irregular divisions of cells of the ground tissue, resulting in the displacement of the primary xylem and phloem groups (Fig. 2B–D). This primary thickening growth also takes place in the cortex.

The primary xylem groups consist of relatively small protoxylem elements and large metaxylem elements which give the impression of transfusion tissue. These metaxylem elements do not only differentiate in a centripetal direction, but also in a lateral and centrifugal direction to form arcs radiating from the protoxylem. Worsdell (1897) also commented on the extension of the centripetal xylem towards the sides of the bundle in the cotyledonary bundles of *Cycas* and *Ginkgo*, in his attempts to determine the origin of the transfusion tissue in Gymnosperm leaves.

The later-formed metaxylem elements at first lie in direct contact with the other metaxylem elements but can at a later stage become separated from them by the intrusion and divisions of the pith parenchyma cells (Fig. 5A).

In the seedling with the octarch root (Fig. 2A) xylem strands 1 and 7 dichotomise at levels C and D respectively, but branch la disappears again at level D and branch 7a disappears at level E. At level F, branches 1b and 7b fuse while 3 and 6 disappear so that at the lower end of the hypocotyl at level G only branches 2, 4, 5 and 7b remain.

In tetrarch roots, the primary xylem strands remain unchanged up to level G, while in the case of a triarch root, one of the xylem strands dichotomises to form a tetrarch stele in the hypocotyl. Pearson (1898) describes a reduction of the pentarch structure to a triarch in *Bowenia* while Dorety (1919), in her description of the seedling of *Dioon spinolosum*, states that ''the vascular cylinder of the hypocotyl is a protostele; it has four easily recognized protoxylem groups, in no way differing from the hypocotyl cylinders of *Ceratozamia* and *Microcycas*''.

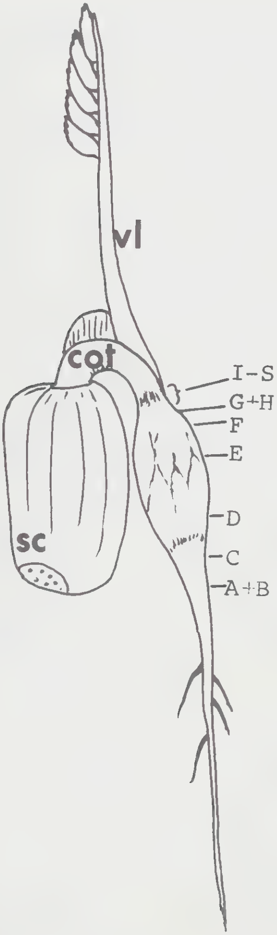


FIG. 1.
Young seedling of *Encephalartos eugene-maraisii* showing levels A–B where sections were made. cot—cotyledon; sc—seed coat; vl—vegetative leaf.

About the seedling of *Dioon edule*, Thiessen (1908) states that at each corner of the squarish vascular plate there is a group of protoxylem elements. In *E. transvenosus* the vascular cylinder is pseudotetrarch. It seems, therefore, that regardless of the number of protoxylem strands in the roots of most of the Cycad seedlings investigated, the hypocotyl is tetrarch or triarch. The triarch condition in *Bowenia* will have to be re-investigated as the condition in *E. eugene-maraisii* at level H (Fig. 2), which resembles that in *Bowenia* to a certain extent, could be misinterpreted as triarch.

The hypocotyl is very short and stretches only from levels G to about K, a length of approximately one millimeter. This is in agreement with what has been found in other Cycad seedlings.

Each of the two cotyledons is supplied with three leaf traces. Traces a, b and c (Fig. 2L) enter the one cotyledon while traces d, e and f (Fig. 2M) enter the second (see also Fig. 3). The central traces *b* and *e* remain unbranched while the lateral traces *c, d* and *f* dichotomise and *a* splits up into three branches (Fig. 2L–O). In the other seedlings of *E. eugene-maraisii* investigated, trace *a* splits up into two branches.

Three leaf traces (strands) were also found in *Cycas revoluta* (Worsdell, 1898), *Cycas siamensis* (Matte, 1904), *Ceratozamia* (Dorety, 1908), *Macrozamia spiralis* (Worsdell, 1898) and *Stangeria paradoxa* (Worsdell, 1898) while in *Dioon edule* (Thiessen, 1908) and *Dioon spinolosum* (Dorety, 1919) two leaf traces are given off to each cotyledon but, before entering the cotyledons, they branch so that four vascular strands enter each cotyledon. In *Zamia muricata* (Karsten, 1856) and *Bowenia spectabilis* (Pearson, 1898) only one leaf trace per cotyledon is given off but it also branches so that each cotyledon is supplied by four vascular strands. According to Coulter & Chamberlain (1910) the condition in *Dioon* is characteristic of the Cycads but from the cited examples it is clear that the three leaf traces per cotyledon as described in this paper for *Encephalartos* is more common among the Cycads. It would seem, therefore, that the seedling of *E. barteri* with three cotyledons which was described by Matte (1904) must have been abnormal. Three leaf traces were also found in a seedling of *E. transvenosus* which was studied as a comparison with seedlings of *E. eugene-maraisii* (Fig. 4).

In his description of the seedlings of *Cycas revoluta, Macrozamia spiralis* and *Stangeria paradoxa*, Worsdell (1898) mentioned three peculiar strands leaving the stele in the hypocotyl and extending ''perpendicularly'' in the cortical tissue. He could not decide whether they were strands of roots or not. In this study similar strands were observed but they were definitely the strands of the so-called apogeotropical roots which later dichotomised to form coralloid roots (Fig. 4, see also Fig. 5D for gaps where these strands leave the central cylinder).

Four vascular bundles enter the first vegetative leaf at two different levels. Bundles *h* and *i* which remain unbranched, have their origin at level O (Fig. 2). Both are compound bundles receiving traces from a common vascular arc between two of the original vascular strands of the hypocotyl. Bundles *g* and *j* originate at level M on the side of the leaf base opposite to the root poles 2 and 4 (Fig. 3) forming a girdle around the vascular plate. The bundles *g* and *j* each divide into four bundles which enter the leaf base at levels P and Q (Fig. 2C). The first

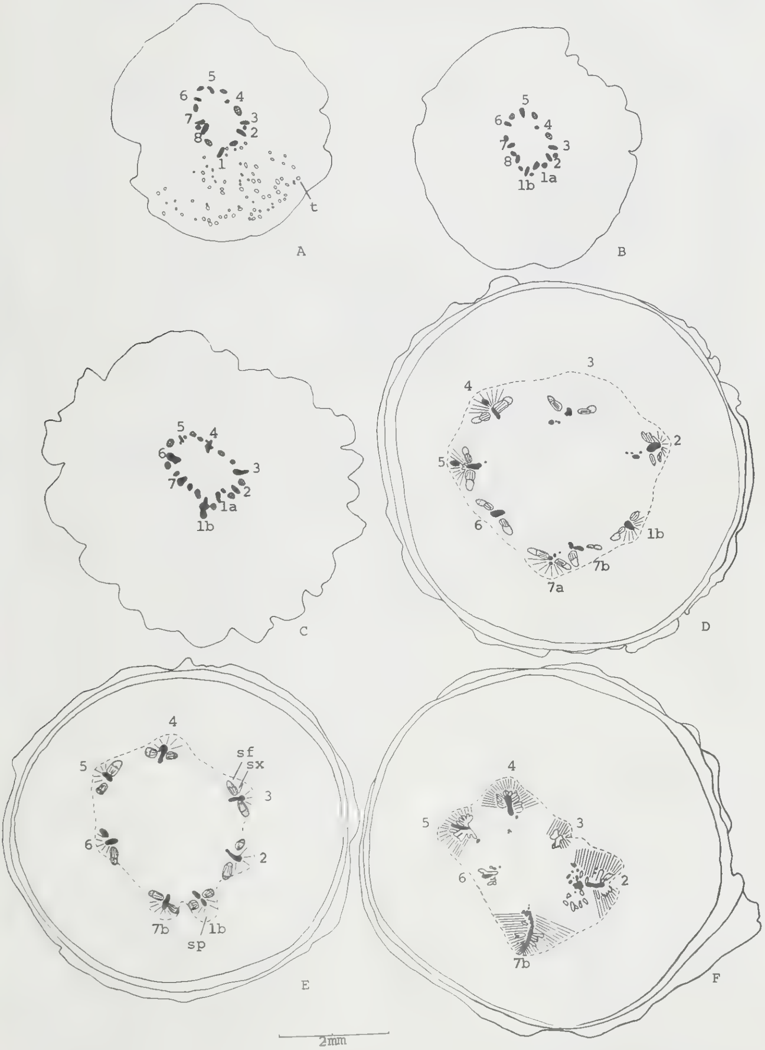
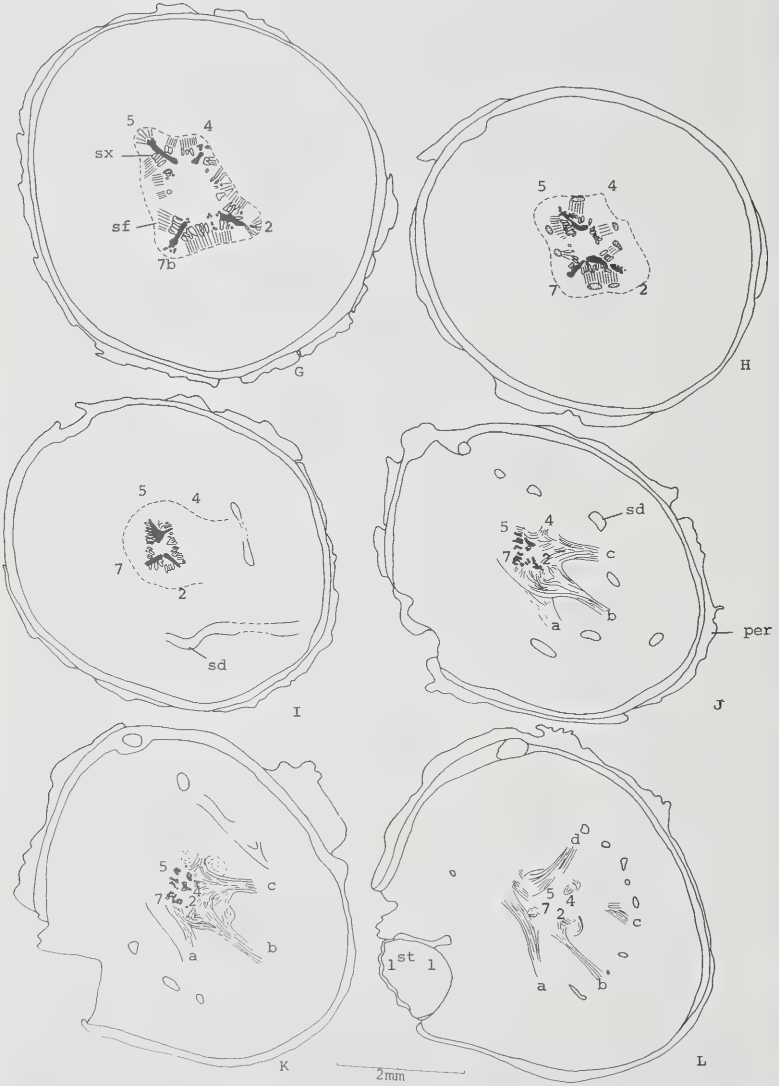


FIG. 2

Serial transverse sections through a seedling of *E. eugene-maraisii* at levels indicated in Fig. 1. 1–8: primary xylem groups in root and hypocotyl. a–f: vascular strands supplying the cotyledons. g–j: vascular strands supplying the first vegetative leaf. am—apical meristem; cot 1 and cot 2—basis of cotyledons; per—periderm; sd—slime ducts; sf—secondary phloem; sp—secondary parenchyma; sx—secondary xylem; t—tanniniferous cells; vpl—vascular plate; 1stl—first vegetative leaf; 2ndl—second vegetative leaf.



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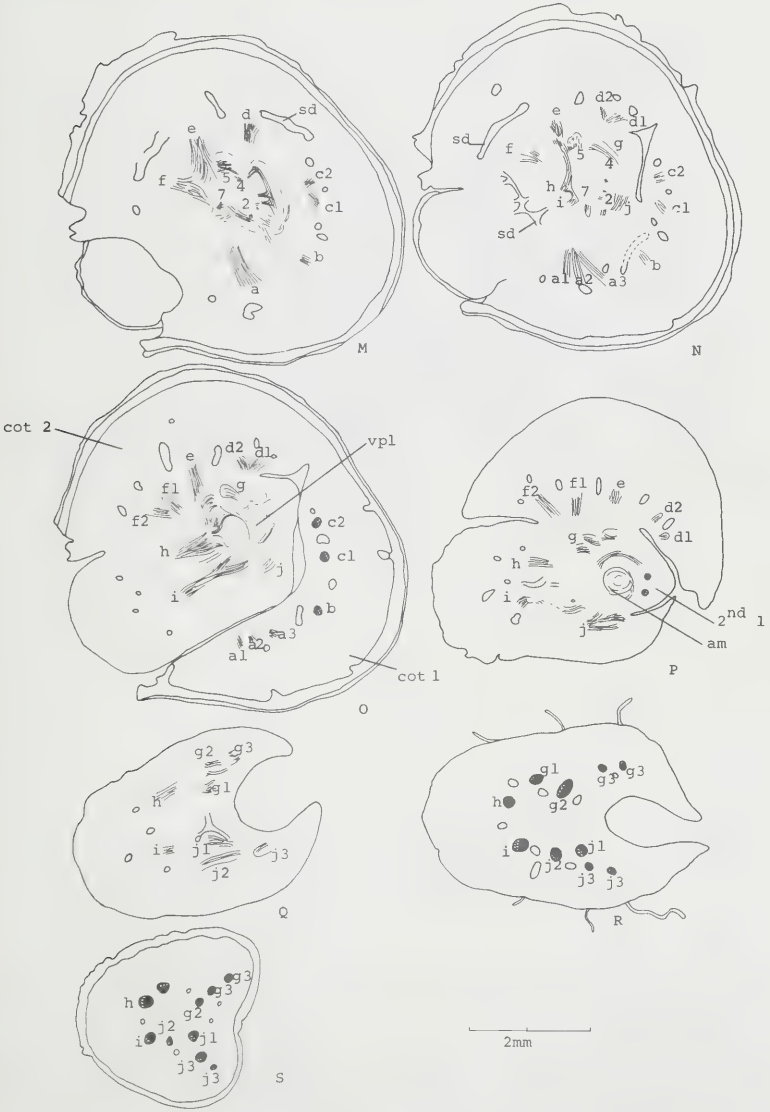

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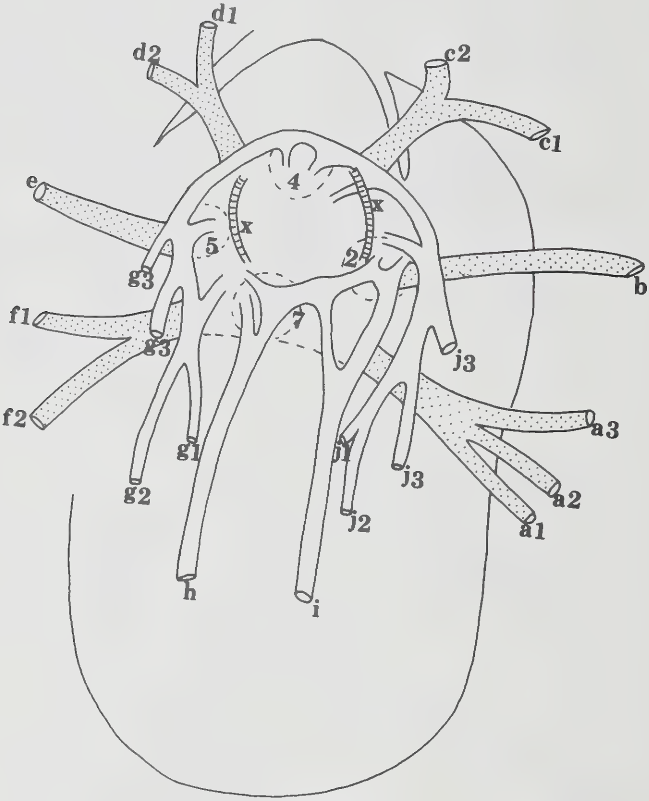

FIG. 3.
Reconstruction of sections A–S in Fig. 2 to illustrate the vasculature in the seedling of *E. eugene-maraisii*. 2, 4, 5 & 7: vascular groups of tetrarch stele. a–f: vascular strands supplying the cotyledons. g–j: vascular strands supplying the first vegetative leaf. x: girdle of vascular tissue supplying the 2nd vegetative leaf.

vegetative leaf thus has a total of ten vascular strands in its base (Fig. 2CR). In *E. transvenosus* (Fig. 4) the same basic pattern is followed.

In his description of the vascular anatomy of the seedling of *Dioon edule*, Thiessen (1908) found four vascular bundles entering the first vegetative leaf. A similar condition occurs in *E. eugene-maraisii* with the exception that in *E. eugene-maraisii* the first two bundles are compound, whereas in *Dioon* they are not. Dorety (1908) also found a similar four-bundle pattern in *Ceratozamia*.

Secondary growth in the root commences when cambium segments are

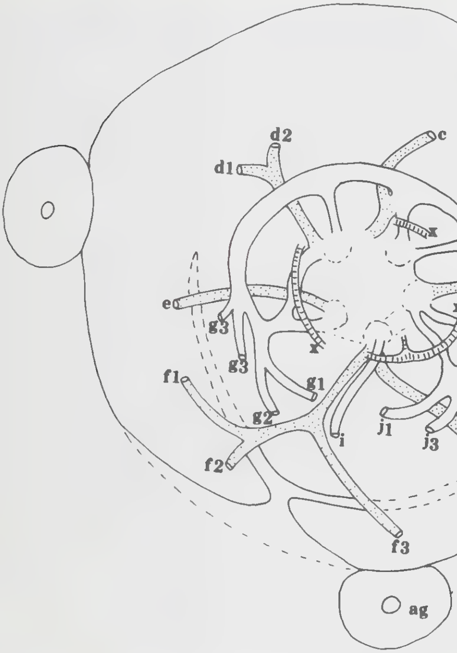

FIG. 4.
Reconstruction of the vasculature in the seedling of *E. transvenosus*. Figures and numbers as for Fig. 3. ag—apogeotropic roots.

differentiated on either side of each radiating primary xylem strand, thus forming secondary tracheids and sieve cells in a more or less tangential direction (Figs 2F and 5C). Because secondary tracheids are about the same size and have the same staining qualities as the metaxylem elements, it is very difficult to differentiate between them. Opposite the protoxylem, a few secondary tracheids may be formed although most of the secondary tissue at this point consists of secondary parenchyma, in broad medullary rays (Fig. 5B).

Le Goc (1914) pointed out that the parenchymatous band between the protoxylem and centrifugal xylem in the petiole of Cycadales is an indication that the centrifugal xylem is of secondary origin. In this study, however, it has been found that even in the root, the metaxylem elements are often separated from the protoxylem by the intrusion of parenchyma cells as a result of primary thickening growth of the tuberous roots (Fig. 5A). The presence of the parenchymatous band, therefore does not provide sufficient evidence for making deductions as to the origin of the centrifugal xylem.

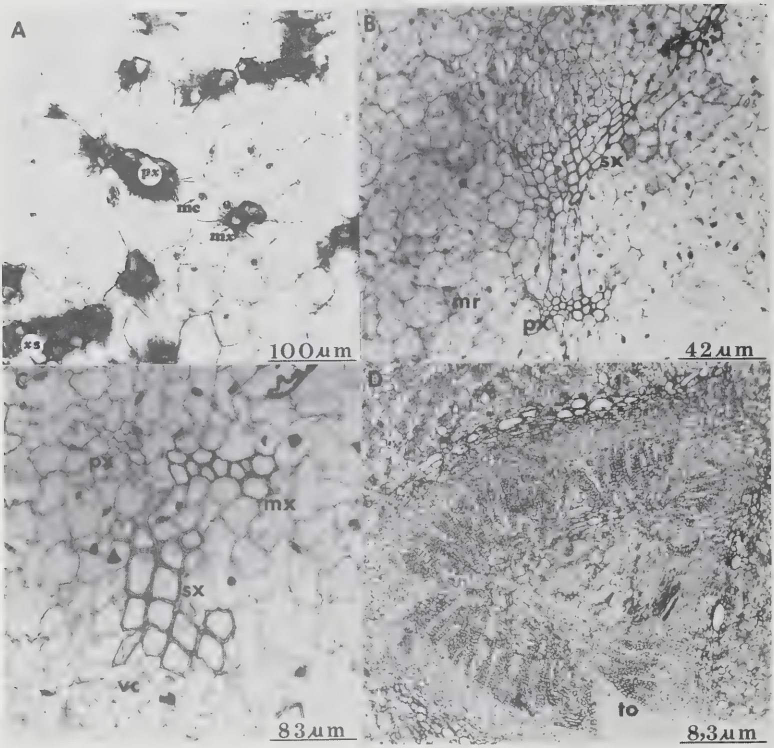

Fig. 5.

Transverse sections at different levels of the seedling of *E. eugene-maraisii.* A at level B in Fig. 2; B at level F in Fig. 2; C at level D in Fig. 2; D from another seedling at level H. mc—meristematic cortical cell; mr—medullary ray parenchyma; mx—metaxylem tracheids; px—protoxylem; sx and xs—secondary xylem tracheids; to—trace gaps for apogeotropical roots; vc—vascular cambium.

When the differentiation of xylem elements from the procambium strands in the first vegetative leaf is followed acropetally from the leaf base into the petiole, it is clear that the position of the protoxylem in the procambium strand in these two regions differs. In the leaf base the first protoxylem elements differentiate near the centre of the procambium strand (Fig. 6A), while the metaxylem elements differentiate centrifugally as well as centripetally, thus giving rise to mesarch xylem (Fig. 6C). Acropetally the first protoxylem elements are gradually displaced centrifugally so that less centrifugal and more centripetal xylem is formed until, near the first pinna, no centrifugal xylem differentiates at all, thus giving rise to

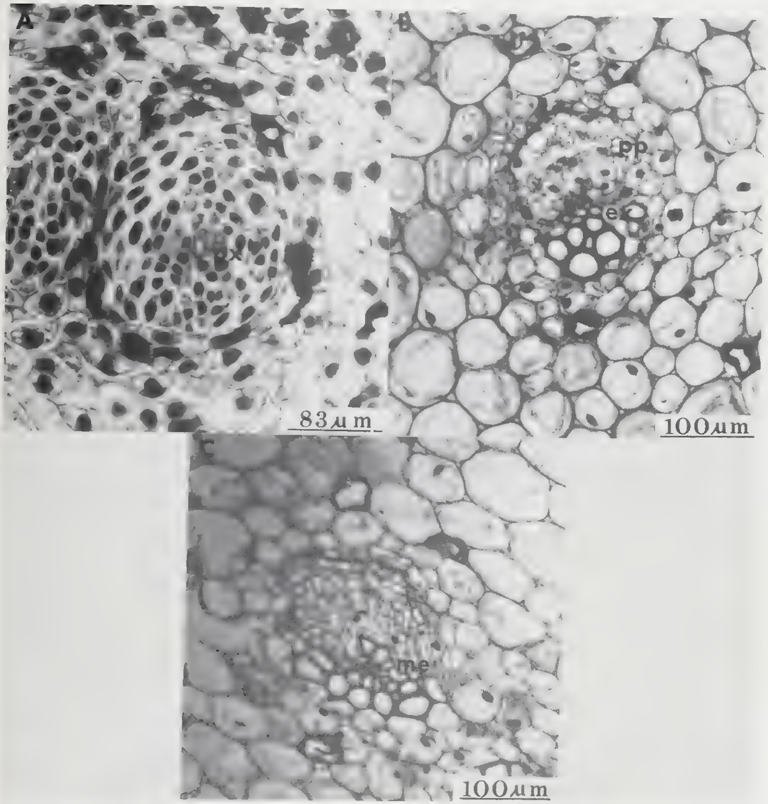

FIG. 6

Segments of transverse sections of the young petiole of *E. eugene-maraisii* to show A—procambium strand with first protoxylem elements in the leaf base; B—exarch xylem near first pinna; C—mesarch xylem in middle of petiole. px—protoxylem elements; pp—primary phloem; ex—exarch xylem group; me—mesarch xylem group.

exarch xylem (Fig. 6B). After secondary thickening growth has commenced, therefore, the centrifugal xylem in the leaf base would consist of metaxylem and secondary xylem whereas in the distal end of the petiole it would consist purely of secondary xylem.

Le Goc (1914) stated that ". . . the centripetal xylem is an independent tissue, probably the remnant of an ancient and more developed structure". He also stated that the centripetal and centrifugal xylem ". . . are continuous physiologically but not morphologically", probably having the parenchyma cells between the protoxylem and the centrifugal xylem in mind. If this was true it would mean the centrifugal xylem would consist only of metaxylem and it would be hard to believe that it could be an ''independent tissue''.

It is therefore concluded that:

1. The primary centripetal and centrifugal xylem has a common group of protoxylem elements and therefore cannot be regarded as independent tissues. Instead it is found in vascular bundles with mesarch primary xylem.
2. Before secondary growth has commenced, the centrifugal xylem consists only of metaxylem elements but it can be supplemented by secondary xylem once secondary thickening growth has taken place.

ACKNOWLEDGEMENTS

This research was sponsored by the Division of Nature Conservation of the Transvaal Provincial Administration and the University of Pretoria. Thanks are due to Mrs J. E. Lombard for reading the manuscript.

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